White paper | June 2022

Precision medicine for non-small cell lung cancer (NSCLC):

Emerging trends in molecular analysis

About the author

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Rania Gaspo earned her B.Pharm and Ph.D. at the University of Montreal, Faculty of Pharmacy. She served as a post-doctoral fellow at Montreal Heart Institute before working as a senior scientist at Merck Research Laboratories. Rania then joined Pfizer where she held positions of increasing responsibilities in medical affairs, clinical development, and medical communications in various therapeutic areas, most notably in oncology. She has also supported the launch of many novel medications, led a global team of experienced medical communications managers, and authored more than 25 peer reviewed publications and 50 scientific communications.

Of all types of cancer, lung cancer caused more deaths worldwide than any other type of cancer.[1] It also causes more deaths than breast and colorectal cancers combined.[1] Patients with the most common lung cancer, non-small cell lung cancer (NSCLC), have higher survival rates than patients with small-cell lung cancer (SCLC), but the outcomes for both remain bleak. According to the National Cancer Institute, the five-year survival rate for NSCLC between 2004 and 2010 was 20.7% compared to only 6.3% for SCLC.[2]

NSCLC patients also have more promising and more precise, treatment options today compared to even 10 years ago. Advances in biomarker and precision medicine have led to the development of immunotherapies and targeted novel treatments that have the potential to improve patient outcomes. In the United States, the FDA has approved dozens of biomarker-driven therapies for NSCLC, including ALK, EGFR, and **ROS1** inhibitors.[3] Meanwhile, more than 1,200 treatments are in development.[4]

With precision medicine becoming a more integral component of NSCLC treatment, molecular testing is an important step to help researchers, pathologists, and oncologists understand the genetic underpinnings of this disease. Pathologists commonly use immunohistochemistry (IHC) and/or next-generation sequencing (NGS) to characterize lesions and refine diagnoses, while circulating tumor DNA (ctDNA) analysis has emerged as a promising, non-invasive companion diagnostic.

This white paper explores the roles IHC, NGS, and ctDNA play in NSCLC precision medicine therapy development, as well as how they help healthcare providers determine the most effective treatment regimens for their patients.

The need for broad molecular profiling

National Comprehensive Cancer Network (NCCN) Clinical Practice Guidelines recommend biomarker testing for patients with newly diagnosed stage IV NSCLC. Oncologists can also consider biomarker testing for patients with squamous cell histology.[5]

NCCN Guidelines suggest broad molecular profiling of multiple biomarkers to identify patients who may benefit from targeted therapies. One of the first steps is testing for biomarker alterations: *EGFR, ALK, ROS1, BRAF, KRAS, NTRK1/2/3, MET, RET,* **and PD-L1.** Most of these genetic mutations or rearrangements have approved targeted or immunotherapies available and/or clinical trial options.[5]

Understanding programmed death-1/programmed-death-ligand 1 (PD-1/**PD-L1**) levels is especially integral to treatment planning. First-line treatment strategies exist for patients with both negative and positive **PD-1/PD-L1** expression, including combinations of surgical intervention, chemotherapy, radiation, and PD-1/PD-L1 inhibitors.[6]

Immunohistochemistry: A cost-effective screening method for biomarker detection

Pathologists across the globe use IHC to identify and quantify biomarkers for precision medicine treatment and research. Many cancer centers choose conventional, single marker IHC stains for their simplicity, speed of execution, and cost-effectiveness. However, multiplex IHC offers the following additional benefits for NSCLC precision medicine treatment and research.

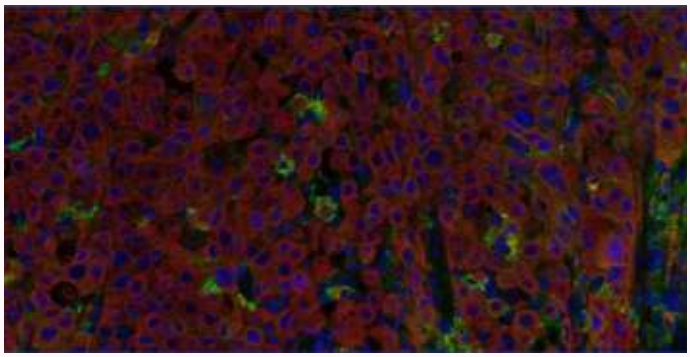
- **Patient-centric approach.** View and review multiple biomarkers on one slide - no need for patients to undergo additional invasive, often painful rebiopsies.
- **Verify co-expression and spatial organization.** View multiple targets within a preserved tissue architecture.
- **Immune profiling.** Characterize tumors and identify predictive biomarkers for immunotherapy response.
- **Move from preclinical to clinical.** Use multiplex IHC to validate targets, select patients, and characterize efficacy and response. This creates an iterative feedback loop where the ability to predict responses from IHC improves as healthcare providers incorporate the data associated with patient outcomes.

Preferred IHC panels for NSCLC

For efficient NSCLC analysis, Cerba Research recommends two immuno-oncology panels:

- the checkpoint inhibitor (CKI) multiplex panel, which consists of CD3, **CD8, PD-1, PD-L1, Custom marker;**
- the PD-L1 multiplex panel, which consists of **CD68, panCK, and PD-L1**.

Both panels contain druggable targets and have been appropriately validated on lung specimen. Then, based on the results of these panels, clinical trialists can design a treatment regimen that's optimally targeted to the patient's makeup and cancer type.



PD-L1 multiplex panel. NSCLC

Available IHC Immuno-oncology multiplex panels at Cerba Research

- T reg (**CD3/CD4/CD8/CD25/FoxP3**)
- T reg light (**CD3/CD8/FoxP3**)
- M1/M2 (C**D68/CD163/c-maf/ pSTAT1)**
- CKI (CD3/CD8/**PD-1/PD-L1/Custom**) **marker**
- PD-L1 panel (**CD68/panCK**/PD-L1)
- Tumor temp (**CD3/CD8/**Tumor mask) –Chromogenic or Fluorescent
- TRM (**CD3/CD8/CD103/CD69/CD49a**)

Selected examples of available lung biomarkers at Cerba Research in IHC

PD-1, PD-L1, ALK, IL-1α, Nkp46, **FoxP3, SIRPa**, Treg*, MDSC*, NK cells*

Bold = Targetable Biomarker, * = Multiplex Assay

Next-generation sequencing: Broad-panel technique (Oncopanels)

Using NGS, pathologists and researchers observe the order of nucleotides in targeted DNA or RNA regions or the entire genome of both germline and cancer cells, depending on the scope of the investigation or study, from biomarker research to identification of actionable mutations. When developing targeted NSCLC therapies, NGS allows researchers to detect important biomarkers with high throughput, scalability, and speed. Cerba Research can typically turn around an NGS broad-panel assay for NSCLC, aka oncopanel, in 10 to 15 days with a relevant and easy-to-read report. This level of efficiency allows patients to start receiving treatment as fast as possible - critically important when you are studying an advanced disease with a low survival rate.

NCCN Guidelines recommend NGS broad-panel testing (e.g. oncopanels) as one of the preferred methods to detect genetic alterations and oncogenic driver mutations. Examples of alterations in NSCLC include *RET*gene fusions *METexon 14* skipping, and *NTRKT1/2/3* gene fusions. *EGFR* is one example of a well-known driver mutation. Other mutations detected by NGS include *BRAF* and several other exploratory biomarkers such as *HER2*. The oncopanel is one of the most common NGS testing method for detecting these gene alterations and mutations in clinical practice.[5]

With patients' genetic information, clinical trialists can recommend effective drugs already available or steer patients to appropriate clinical trial options. Until recently, NGS was reserved for reference laboratories and large cancer centers due to cost. Now that the cost to implement NGS has dropped, more hospitals are bringing the technology in-house.[7] Although generally more expensive and less accessible than IHC, both techniques ideally complement themselves to give a full characterization of the tumor.

NGS use case

A physician performs a biopsy to obtain a sample of a patient's lung lesion. A molecular pathologist and his team run the sample through a broad-panel NGS validated assay– either DNA, RNA, or both. The result demonstrates the patient suffering from metastatic NSCLC has an *ALK* rearrangement. The physician may opt to prescribe Ceritinib, an *ALK* inhibitor approved for use in metastatic NSCLC.[8]

Most commonly deployed techniques for NSCLC biomarkers[5]

Biomarker	Most commonly deployed techniques	Additional techniques
EGFR	NGS	RT-PCR. Sanger sequencing
ALK	NGS, IHC	FISH, Liquid Biopsy
ROS1	NGS	FISH,IHC
BRAF	NGS	RT-PCR, Sanger sequencing
KRAS	NGS	
MET	NGS	
RET	NGS	FISH, RT-PCR
NTRK1/2/3	NGS, IHC	FISH, PCR
EGFR T790M	NGS	Liquid Biospy
PD-L1	IHC	

On the horizon: Circulating tumor DNA (liquid biopsy)

One of the newer testing methods in oncology, ctDNA, is attracting significant attention due to its non-invasive nature and practicality for early detection, ongoing monitoring, and rapid treatment.[9,10]ctDNA refers to cancer cell DNA that breaks down and releases into the bloodstream. Analyzing genetic alterations and mutations using a ctDNA approach, aka liquid biopsy, helps reduce the need for an invasive tissue biopsy or rebiopsy.

Aside from the ability to obtain genetic information via a blood draw, ctDNA testing offers the following advantages:

- **Serial sampling:** Use in conjunction with or without tissue samples
- **Ease of use:** Requires less staff to implement
- **Patient comfort:** Patients can provide a sample during an outpatient visit
- **Minimally Invasive:** Reduces the need for biopsy or rebiopsy
- **Tissue is the issue:** 1/5 patients don't have enough tissue
- **Monitor:** Sometimes used to monitor tumor burden longitudinally.

Currently, ctDNA diagnostics have just started providing robust specificity and sensitivity. Researchers; however, continues to work hard in improving the performance and clinical utility of these techniques to meet or exceed IHC and NGS in both dimensions. In addition, ctDNA diagnostics continues to be expensive, reserving the assay to large cancer centers or central laboratories such as Cerba Research. However, the cost effectiveness and clinical utility is expected to improve over time, much like NGS for tissue biopsy.

Cerba Research services for NSCLC

With fully equipped histopathology services across the U.S., Europe, and Asia, Cerba Research offers the technology and expertise for expedited NSCLC diagnostics. Serving preclinical to clinical research, Cerba Research has over 200 IHC biomarkers/protocols available and a biobank with over 3,000 blocks available and growing. We also develop and validate custom biomarkers according to clinical trial needs. We perform both conventional and multiplex IHC, with the ability to detect up to nine biomarkers in one multiplex IHC panel. An international network of board-certified pathologists and consultants analyzes IHC results.

Cerba Research also offers global NGS capabilities with multiple comprehensive oncopanels available for analysis. Our capacity for high throughput, with the ability to sequence more than 1,000 whole human genomes in as little as 10-15 days, provides the information needed to bring targeted medicines to patients faster. We are also one of the few diagnostics solutions providers to offer ctDNA analysis. CerbACT Asia, one of our ImmunoOncology Centers of Excellence, offers multiple ctDNA-based assays using the ACTMonitor® platform. Cerba Research Paris offers the EGFR T790M assay on liquid biopsy and many additional NGS oncopanels according to your clinical trial needs. Cerba Research Paris is also currently validating relevant large oncopanels for NSCLC and solid tumors on both FFPE and Liquid Biopsy.

Finally, with Cerba Research, you will have access to:

- A worldwide network of over 800 laboratories operating more than 70 technical platforms
- Over 700 clinical pathology experts in several therapeutic areas (oncology, metabolism, endocrinology, toxicology, genetics, autoimmunity, allergology, pregnancy, infectious disease, neurodegenerative disorders, cardiology, hematology) providing access to patients samples, patients recruitment, and ability to build prospective cohorts
- Broad range of 3,000 analysis and more than 1,100 instruments available
- A dedicated scientific team to support you from end to end of your project
- An international network of board certified pathologists and consultants involved in IHC scoring, QC and more as aligned with regulatory authorities

Conclusion

As researchers identify more druggable targets through highly specific, sensitive testing methods such as IHC, NGS, and ctDNA, we expect the development of targeted therapies for lung cancer to progress rapidly and for patient care to improve incrementally. As the process quickens, patients living with this devastating disease will have greater potential to live higher quality and hopefully progression-free lives.

A Cerba Research snapshot in NSCLC precision medicine

DNA

- NGS
- Oncopanels
- Custom panels
- ctDNA
- DNA extraction
- Strek tubes
- ddPCR
- qPCR
- Whole exome
- Whole genome

Tissue

- Multiplex/Simple IHC
- 200+ biomarkers/protocols
- Centralized pathology reading
- Large biobank
- FISH, ISH protocols
- Strong immuno-onco simplex & multiplex panels
- Full histopath service
- Spatial analysis in the tumor microenvironment

RNA

- RNAseq (fusion genes)
- NGS
- Oncopanels
- Custom panels
- rtPCR
- PaxGene®
- Nanostring partnership

Cell

- FCM panels
- NGF Cytek Aurora under dev (up to 40 colors)
- Immunophenotyping
- Lymphocyte infiltration
- Marker analysis (cell surface/cytoplasmic)

Protein

- Electrophoresis
- Multiplex cytokine profiling
- Custom ligand binding assays–ELISA

About Cerba Research

Cerba Research, a strategic provider of diagnostic solutions, supports drug development by leveraging patient data and scientific insight to optimize R&D and commercialization globally. Providing early phase research, clinical development through central laboratory and diagnostic testing, assay and biomarker development and validation – through our global network of specialty laboratories. We partner with government agencies, non-government organizations, as well as pharma and biotech organizations to change the shape of clinical development.

Cerba Research is part of Cerba HealthCare, a leading player in medical diagnosis.

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